

--13. A method of producing a hybrid DNA molecule having a sense strand and an anti-sense strand and in which, reading in the 5' to 3' direction, the sense strand has the sequences x_1, x_2, \dots, x_n , where n is greater than or equal to 3, the method comprising the steps of

- (i) providing in a single reaction mixture
 - (a) the sequences x_1, x_2, \dots, x_n and their complementary sequences x_1', x_2', \dots, x_n' , to be assembled into the hybrid molecule,
 - (b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers for the 3' ends of any two sequences ($x_i, x'_{(i+1)}$), where i is 1 to $(n-1)$, have specifically complementary linker sequences connected to their respective priming sequences via an adenine residue, and
- (2) effecting a first stage PCR reaction in which those primers provided with linker sequences are present in limiting concentrations and a polymerase which adds a 3' adenine overhang to the end of an extended strand; and
- (3) effecting a second stage PCR reaction using a single pair of primers one of which provides the 5'-end of the sense strand and other of which provides the 3'-end of the anti-sense strand of the required hybrid molecule

whereby said hybrid molecule is generated.

14. A method as claimed in claim 13 wherein the polymerising enzyme for steps (2) and (3) is *Taq*.

15. A method as claimed in claim 13 wherein the annealing temperature (T_m) of the linker sequences is greater than that of the priming sequences to the x and x' sequences.

16. A method as claimed in claim 15 wherein the annealing temperature of the linker sequences is 2 to 5°C greater than that of the priming sequences to the x and x' sequences.

17. A method as claimed in claim 13 wherein the linker sequences do not have intrinsic secondary structure.

18. A method as claimed in claim 13 wherein between the first and second stage PCR reactions the reaction mixture is frozen to deactivate residual PCR activity.

19. A method as claimed in claim 13 wherein between the first and second stage PCR reactions the reaction mixture is treated with an exonuclease I to digest single stranded molecules.

20. A method as claimed in claim 13 wherein each of the first and second stage PCR reactions utilise a thermally activated polymerase.

21. A method of mutation analysis wherein the analysis is effected on a DNA hybrid molecule produced in accordance with the method of claim 1.

22. A set of primers incorporating the following sequences.

5'tcatattagccgctgcattgcc-a-3'

5'ggcaatgcagcggctaataa-a-3'

5'agccactacccaaactcctgt-a-3'

5'acaggagtttggttagtgct-a-3'

5'tgtctcactgaacctgcctacct--a-3'

5'aggtaggcaggttcagttagaca-a-3'

5'cctcat taccggctgtcagactg-a-3'

5'cagtctgacagccgtaataagg-a-3'